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(54) Title: METHOD OF PRODUCING PLANTS WHICH ARE TOLERANT OR RESISTANT TO HERBICIDES

(57) Abstract

A method of making plants which are resistant or tolerant to herbicides which, *in vitro*, inhibit 4–hydroxyphenylpyruvate dioxygenase (4HPPD) comprises the steps of: (i) transforming plant material with a polynucleotide comprising a region encoding a phytoene desaturase; (ii) regenerating the thus transformed material into morphologically normal plants. In a preferred embodiment the region comprised by the polynucleotide is the sequence depicted in SEQ ID No.1, or is a sequence which is complementary to one which when incubated at a temperature of between 55 and 60 °C in 0.3 strength citrate buffered saline containing 0.1 % SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1 % SDS still hybridises with the sequence depicted in SEQ ID No.1.

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METHOD OF PRODUCING PLANTS WHICH ARE TOLERANT OR RESISTANT TO HERBICIDES

The present invention relates *inter alia*, to a method of producing plants which are tolerant or resistant to herbicides and in particular to the production of transgenic plants which exhibit substantial resistance or substantial tolerance to herbicides when compared with non transgenic like plants.

Plants which are substantially "tolerant" to a herbicide when they are subjected to it provide a dose/response curve which is shifted to the right when compared with that provided by similarly subjected non tolerant like plants. Such dose/response curves have "dose" plotted on the x-axis and "percentage kill", "herbicidal effect" etc. plotted on the y-axis. Tolerant plants will require more herbicide than non tolerant like plants in order to produce a given herbicidal effect. Plants which are substantially "resistant" to the herbicide exhibit few, if any, necrotic, lytic, chlorotic or other lesions when subjected to the herbicide at concentrations and rates which are typically employed by the agrochemical community to kill weeds in the field. Plants which are resistant to a herbicide are also tolerant of the herbicide. The terms "resistant" and "tolerant" are to be construed as "tolerant and/or resistant" within the context of the present application.

The herbicides of particular relevance to the present invention are those which are capable *in vitro* of inhibiting 4-Hydroxy-phenylpyruvate dioxygenase (HPPD or 4HPPD) enzymes. Such herbicides have been disclosed, such as the isoxazoles described especially in the French Patent Applications 95 06800 and 95 13570 and especially isoxaflutole, a selective maize herbicide, diketonitriles such as those described in European Applications 0 496 630, 0496 631, in particular 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-CF_3-phenyl)propane-1,3-dione and 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-2,3Cl_2phenyl)propane-1,3-dione, triketones described in European Applications 0 625 505 and 0 625 508, in particular sulcotrione, mesotrione (BSI-proposed), pyrazolynate and pyrazoxyfen. Known genes capable of providing for tolerance to these herbicides are those which encode HPPD enzymes.

According to the present invention there is provided a method of making plants which are resistant or tolerant to herbicides which - *in vitro* - inhibit 4-hydroxyphenylpyruvate dioxygenase (4HPPD) comprising the steps of:

(i) transforming plant material with a polynucleotide comprising a region encoding a phytoene desaturase (PDS);

(ii) regenerating the thus transformed material into morphologically normal plants.

The region comprised by the polynucleotide may have the sequence depicted in SEQ ID No. 1, or may be a sequence which is complementary to one which when incubated at a temperature of between 55 and 60°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS still hybridises with the sequence depicted in SEQ ID No. 1.

It is preferred that the phytoene desaturase is of bacterial origin such as that depicted in SEQ ID No. 1 and being derived from *Erwinia uredovora*, and/or in particular is one which does not require plastoquinone 9 as a co-factor. The desaturase may, however be of plant origin, such as especially of monocotyledonous or dicotyledonous plants, especially of *Arabidopsis* or of *Umbelliferae*, such as, for example, the carrot (*Daucus carotta*). It can be native or possibly mutated while at the same time fundamentally retaining a property of herbicidal tolerance against HPPD inhibitors, such as herbicides of the isoxazoles family such as the Balance ™ Herbicide or triketones. The herbicide resistant plants produced by the above method may be selected through their resistance to herbicides which *in vitro*, inhibit 4HPPD. In may however, be further preferred that the polynucleotide encoding the phytoene desaturase further comprises a selectable marker gene to facilitate the selection of regenerated transformats. Suitable selectable marker genes include; resistance to antibiotics such as kanamycin, hygromycin and gentamycin; resistance to further herbicides such as glyphosate based herbicides; resistance to toxins such as eutypine.

Other forms of selection are also available such as hormone based selection systems such as the Multi Auto Transformation (MAT) system of Hiroyrasu Ebinuma *et al.* 1997. PNAS Vol. 94 pp2117-2121; visual selection systems which use the known green flourescence protein, β glucoronidase, mannose isomerase, xylose isomerase and 2-DOG.

The plant material may be, or may have been, further transformed with a polynucleotide comprising a region encoding a protein capable of providing the plant material with

resistance or tolerance to herbicides, insects, desiccation and/or fungal, bacterial or viral infections, or with a polynucleotide capable of encoding proteins which provide for improved quality traits such as increased yield, altered starch quality and/or increased nutrient content.

The protein encoding sequences within the polynucleotide are bounded by plant operable promoters and terminators. Such promoters and terminators, which are *per se* not germane to the invention, are well known to the skilled man and include, for example, the CaMV35S, FMV35S, NOS, OCS and E9 (derived from the small subunit of RUBISCO) promoters and terminators, or the promoter and terminator of a gene of alpha-tubulin (EP-A 652,286). Preferably, recourse is made to a promoter regulation sequence which favours the over-expression of the coding sequence, such as, for example, that comprising at least one histone promoter such as described in EP-A-507,698.

According to the invention, it is equally possible to use, in association with the promoter regulation sequence, other regulation sequences which are situated between the promoter and the coding sequence, such as transcriptional or translational enhancers such as, for example, tobacco etch virus (TEV) translation activator described in International Patent application, PCT publication number WO87/07644 which is incorporated herein by reference, or of transit peptides, either single, or double, and in this case possibly separated by an intermediate sequence, that is to say comprising, in the transcription direction, a sequence coding for a transit peptide of a plant gene coding for a plastid localization enzyme, a part of the sequence of the N-terminal mature part of a plant gene coding for a plastid localization enzyme, then a sequence coding for a second transit peptide of a plant gene coding for a plastid localization enzyme, formed by a part of the sequence of the N-terminal mature part of a plant gene coding for a plastid localization enzyme, such as described in EP-A-508,909.

The plant material may have been, or may subsequently be - further transformed with a polynucleotide comprising a region encoding a protein capable of providing the plant with resistance or tolerance to herbicides, insects, desiccation and/or fungal, bacterial or viral infections, or with a polynucleotide capable of encoding proteins which provide for improved quality traits such as increased yield, altered starch quality and/or increased nutrient content.

The protein capable of providing for herbicide resistance may be selected from the group consisting of glyphosate oxido-reductase (GOX), 5-enol-pyruvyl-3-phosphoshikimate synthetase (EPSPS), phosphinothricin acetyl transferase (PAT), hydroxyphenyl pyruvate dioxygenase (HPPD), glutathione S transferase (GST), cytochrome P450, Acetyl-COA carboxylase (ACCase), Acetolactate synthase (ALS), protoporphyrinogen oxidase (PROTOX), dihydropteroate synthase, polyamine transport proteins, superoxide dismutase (SOD), bromoxynil nitrilase, the product of the *tfd*A gene obtainable from *Alcaligenes eutrophus,* and known mutagenised or otherwise modified variants of the said proteins.

As indicated above, the polynucleotide with which the plant material may be transformed may comprise 5' of the protein encoding regions regions which encode: (i) a peptide which is capable of targeting the translation products of the regions to plastids such as chloroplasts, mitochondria, other organelles or plant cell walls; and/or (ii) non-translated translational enhancing sequences.

The polynucleotide may be codon-optimised, or otherwise altered to enhance at least transcription once it is incorporated into plant material. Thus the polynucleotide used to transform the material may be modified in that mRNA instability encoding motifs and/or fortuitous splice regions may be removed, or plant preferred codons may be used so that expression of the thus modified polynucleotide in a plant yields substantially similar protein having a substantially similar activity/function to that obtained by expression of the unmodified polynucleotide in the organism in which the protein encoding regions of the unmodified polynucleotide are endogenous, with the *proviso* that if - in respect of the herbicide resistance conferring regions - the thus modified polynucleotide comprises plant preferred codons, the degree of identity between the protein encoding regions within the modified polynucleotide and like protein encoding regions endogenously contained within the said plant and encoding substantially the same protein is less than about 70%.

Transformation techniques are well known and include particle mediated biolistic transformation, *Agrobacterium*-mediated transformation, protoplast transformation (optionally in the presence of polyethylene glycols); sonication of plant tissues, cells or protoplasts in a medium comprising the polynucleotide or vector; micro-insertion of the polynucleotide or vector into totipotent plant material (optionally employing the known silicon carbide "whiskers" technique), electroporation and the like.

The invention still further provides morphologically normal fertile (or male sterile) whole plants regenerated from the material mentioned in the paragraph immediately preceding the last and the progeny of such plants, the seed of such plants and progeny, and parts of such plants and progeny. The transformed inventive plants include small grain cereals, oil seed crops, fibre plants, fruit, vegetables, plantation crops and trees. Particularly preferred such plants include soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, grape, citrus and nut plants.

The transformed plants of the invention have tolerance or resistance to certain herbicides such as the isoxazoles described especially in French Patent Applications 9506800 and 95 13570 and especially of 4-[4-CF3-2-(methylsulphonyl)benzoyl]-5-cyclopropylisoxazole, and especially isoxaflutole, a selective maize herbicide, the diketonitriles such as those described in EP-A-496,630 and EP-A-496,631, in particular 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-CF_3-phenyl)propane-1,3-dione and 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-2,3-Cl_2-phenyl)propane-1,3-dione, and the triketones described in EP-A-625,505 and EP-A-625,508, in particular sulcotrione, mesotrione (BSI-proposed), pyrazolynate and pyrazoxyfen.

The invention further includes a morphologically normal fertile (or male sterile) whole plant resulting from the method of the invention, the progeny of such plants, the seed of such plants and progeny, and parts of such plants and progeny.

The invention still further provides the use of a polynucleotide comprising a region encoding a phytoene desaturase in the production of plant material which is resistant or tolerant to herbicides which - *in vitro* - inhibit the enzyme 4-HPPD.

The invention still further provides a method of selectively controlling weeds in a field, the field comprising weeds and crop plants, the method comprising application to the field of a herbicide which - *in vitro* - is capable of inhibiting the enzyme 4-HPPD, characterised in that the plants have been transformed with and express the coding regions of a polynucleotide comprising a sequence encoding a phytoene desaturase.

It is particularly preferred that the phytoene desaturase encoding sequence is that which is depicted in SEQ ID No. 1, or is complementary to one which when incubated at a temperature of between 55 and 60°C in 0.3 strength citrate buffered saline containing 0.1%

SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS still hybridises with the sequence depicted in SEQ ID No. 1. The herbicide may be selected from the group consisting of mesotrione (BSI-proposed), pyrazolynate and pyrazoxyfen, Balance,™ sulcotrione etc. The field may be treated with a pesticide selected from the group consisting of a fungicide, insecticide and nematicide, either prior to or post application to the field of the herbicide.

The invention will now be described by way of the following non-limiting example, figure and the Sequence Listing in which:

SEQ ID No. 1 is the sequence of the phytoene desaturase (dehydrogenase) gene isolated from *Erwinia uredovora*. The person skilled in the art will recognise that any phytoene desaturase gene may be used in the production of plants having resistance/tolerance to the herbicides described above.

SEQ ID No. 2 is the protein encoded by SEQ ID No 1.

SEQ ID No.3 is the polynucleotide sequence encoding the pea rubisco small subunit transit peptide.

SEQ IN No. 4 is the amino acid sequence encoded by SEQ ID No. 3.

Figure 1 is the structure of plasmid pYPEIT4 carrying the *Erwinia uredovora* crtI gene with the transit peptide sequence (depicted as TP) of the pea rubisco small subunit.

EXAMPLE

Production of plants tolerant to herbicides capable of inhibiting the enzyme 4-HPPD *in vitro*.

The PDS gene (crtI) was cloned from *Erwinia uredovora,* a non-green phytopathogenic bacterial rot, and over-expressed in transgenic tobacco and tomato using a plasmid containing the CaMV 35S promoter and a chloroplast transit peptide (pYPIET4) (Misawa *et al.*, 1993). Homozygous tomato lines over-expressing the crtI gene were obtained as were tobacco plants containing the same construct.

Construction of plasmid pYPIET4 carrying the tp-crtI gene

Recombinant DNA techniques were performed using standard methods. A DNA sequence coding for the transit peptide (TP) in the precursor of the ribulose-1,5-bisphosphte carboxylase (Rubisco) small subunit of pea was isolated from plasmid pSNIF83 (Schreier *et al.*, 1985) as a 204 bp HindIII-Sphl fragment, whose Spgl site contains the tp processing site. Plasmid pCRT-1 (Fraser et al (1992) J.Biol.Chem 267 19891-19895) carrying the intact phytoene desaturase gene (crtI) of *Erwinia uredovora* was digested with BamHl and HindIII, and a 1.57 kb BamHl-HindIII fragment carrying the truncated crtl gene was isolated. The above 204 bp HindIII-Sphl TP fragment was ligated with a 76bp synthesized fragment which carries the reading frame from the cohesive end for the Sphl site containing the crtl initiation codon to that of the BamHl site, and with the 1.57 kb BamHl-HindIII fragment. The desired 1.84 kb *Hin*dlll fragment carrying the tp-crtl chimeric gene was isolated, filled in with Klenow enzyme, and ligated into the Smal-Sacl site of a 10.9 kb fragment removing the β-glucuronidase gene from the binary vector bB/121 (purchased from Clontech laboratories). Thus, the desired plasmid pYPEIT4 was created, shown in Figure 1. The initiation codons for the transit peptide and the intact Crtl are underlined. This HindIII fragment carrying the tp-crtI gene is surrounded by the CaMV 35S promoter and the NOS terminator of the binary vector pBl121 in order to lead to sufficient expression in the tissues of transgenic tobacco and tomato plants. As a control, plasmid pBICAR4 was constructed which carries an intact crtl gene without tp surrounded by the CaMV 35S promoter and the NOS terminator. The plasmid pYPEIT4 was introduced into tobacco and tomato material by known techniques and the material then regenerated into intact plants, again by known techniques.

Tolerance of Tomato Plants transformed with crtI gene to Mesotrione and Isoxaflutole

Homozygous seed of tomato plants cv. Ailsa Craig, derived from 'wild type' (i.e. un-transformed) and plants transformed with the *crtI* gene from *Erwinina uredovora*, (see above) were sown in a peat-based compost in 3 inch pots and transferred to the glasshouse. Plants were grown at 20/16 degrees day/night temperature under a 16 hour photoperiod for approximately 4 weeks prior to post-emergence treatment of four replicates with mesotrione or isoxaflutole (Balance ™ Herbicide) at the 3 leaf stage. The chemicals were suspended in water and applied, via a track sprayer at a spray volume of 200 litres per hectare, at rates ranging from 1 to 500 grammes active ingredient per hectare (g a.i./ha), as shown in Table 1.

The plants were left to grow for a further 25 days and then assessed visually for herbicidal damage compared to untreated 'control' plants. Typical phytotoxic symptoms observed were extreme chlorosis/bleaching and necrosis of leaves and new growth. The results from this test are given in Table 1 below where the '% Damage/Phytotoxicity' scores represent the mean of the visual assessment from each of the four treatment replicates.

Table 1

Chemical	Rate (g a.i./ha)	% Damage/Phytotoxicity (25 days after treatment)	
		Wild Type (Un-Transformed)	Transformed (crtI)
Mesotrione	1 3 11 33	25 29 50 81	0 0 9 49
Isoxaflutole (Balance™)	1 5 15 50 150 500	11 15 19 21 34 65	2 4 6 8 19 31

As can be seen, plants transformed with the crtI gene which expresses the bacterial PDS from *Erwinia uredovora*, demonstrate elevated tolerance to mesotrione and isoxaflutole compared to wild type, un-transformed tomatoes. For example, 11 g a.i./ha of mesotrione caused 50% phytotoxicity to wild type tomatoes but only 9% injury is observed in the transformed plants. Similarly, wild type plants are significantly more damaged by 500 g a.i./ha of isoxaflutole than those containing the crtI gene.

The skilled man will recognise that the invention is not limited to that described above. For example, plants other than tomato and tobacco may be transformed with a gene encoding a PDS enzyme, whether derived from a bacterial source or otherwise.

CLAIMS

1. A method of making plants which are resistant or tolerant to herbicides which - *in vitro* - inhibit 4-hydroxyphenylpyruvate dioxygenase (4HPPD) comprising the steps of:

(i) transforming plant material with a polynucleotide comprising a region encoding a phytoene desaturase (PDS);

(ii) regenerating the thus transformed material into morphologically normal plants and selecting from the population of regenerants those plants which are resistant or tolerant to herbicides which *in vitro* inhibit 4HPPD.

2. A method according to claim 1, wherein the region comprised by the polynucleotide is the sequence depicted in SEQ ID No. 1, or is a sequence which is complementary to one which when incubated at a temperature of between 55 and 60°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS still hybridises with the sequence depicted in SEQ ID No. 1.

3. A method according to claim 1, wherein the phytoene desaturase is of plant origin.

4. A method according to claim 1, wherein the phytoene desaturase is of bacterial origin.

5 A method according to claim 4 wherein the phytoene desaturase is isolatable from *Erwinia uredovora.*

6. A method according to any one of claims 1 to 5 wherein the polynucleotide further comprises a selectable marker gene.

7. A method according to claim 6 wherein the said selectable marker gene is selected from the group consisting of antibiotic resistance conferring, herbicide resistance conferring, toxin resistance conferring, nutritional markers, visual markers and marker genes used in hormone based selection systems.

8. A method according to any one of claims 1 to 7, wherein the plant material has been or is further transformed with a polynucleotide comprising a region encoding a protein capable of providing the plant material with resistance or tolerance to herbicides, insects, desiccation and/or fungal, bacterial or viral infections, or with a polynucleotide capable of encoding proteins which provide for improved quality traits such as increased yield, altered starch quality and/or increased nutrient content.

9. A method according to any one of claims 1 to 8, wherein the protein encoding sequences within the polynucleotide are bounded by plant operable promoters and terminators.

10. A method according to either of claims 8 or 9, wherein the protein capable of providing for herbicide resistance is selected from the group consisting of glyphosate oxido-reductase (GOX), 5-enol-pyruvyl-3-phosphoshikimate synthetase (EPSPS), phosphinothricin acetyl transferase (PAT), hydroxyphenyl pyruvate dioxygenase (HPPD), glutathione S transferase (GST), cytochrome P450, Acetyl-COA carboxylase (ACCase), Acetolactate synthase (ALS), protoporphyrinogen oxidase (PROTOX), dihydropteroate synthase, polyamine transport proteins, superoxide dismutase (SOD), bromoxynil nitrilase, the product of the tfdA gene obtainable from *Alcaligenes eutrophus,* farnesyl pyrophosphate synthase and known mutagenised or otherwise modified variants of the said proteins.

11. A method according to any one of claims 1 to 10, wherein the protein encoding sequences of the polynucleotide comprise 5' regions which encode: (i) a peptide which is capable of targeting the translation products of the regions to plastids such as chloroplasts, mitochondria, other organelles or plant cell walls; and/or (ii) non-translated translational enhancing sequences.

12. A method according to any one of claims 1 to 11, in which the polynucleotide used to transform the material is modified in that mRNA instability encoding motifs and/or fortuitous splice regions are removed, or plant preferred codons are used so that expression of the thus modified polynucleotide in a plant yields substantially similar protein having a substantially similar activity/function to that obtained by expression of the unmodified polynucleotide in the organism in which the protein encoding regions of the unmodified polynucleotide are endogenous, with the *proviso* that if - in respect of the herbicide resistance conferring regions - the thus modified polynucleotide comprises plant preferred codons, the degree of identity between the protein encoding regions within the modified polynucleotide and like protein encoding regions endogenously contained within the said plant and encoding substantially the same protein is less than about 70%.

13. A method according to any one of claims 1 to 12, in which the 4-HPPD inhibiting herbicide is selected from the group consisting of isoxaflutole, diketonitriles such as 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-CF_3-phenyl)propane-1,3-dione and 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-2,3Cl_2phenyl)propane-1,3-dione, triketones such as sulcotrione, and mesotrione (BSI-proposed), pyrazolynate and pyrazoxyfen.

14. A method according to any one of claims 1 to 13, wherein the herbicide is applied post-germination.

15. A morphologically normal fertile (or male sterile) whole plant resulting from the method of any one of claims 1 to 14, the progeny of such plants, the seed of such plants and progeny, and parts of such plants and progeny.

16. A plant according to claim 15 selected from the group consisting of banana, cotton, maize, tomato, vines.

17. Use of a polynucleotide comprising a region encoding a phytoene desaturase in the production of plant material which is resistant or tolerant to herbicides which - *in vitro* - inhibit the enzyme 4-HPPD.

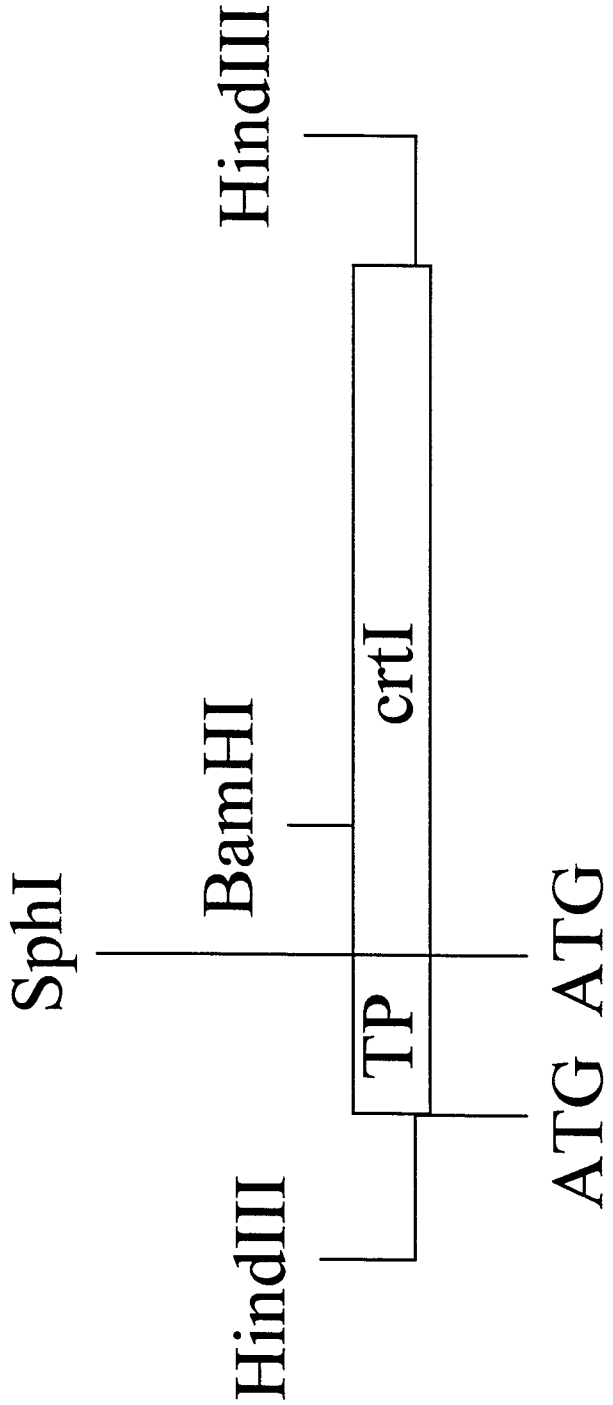
18. A method of selectively controlling weeds in a field, the field comprising weeds and crop plants, the method comprising application to the field of a herbicide which - *in vitro* - is capable of inhibiting the enzyme 4-HPPD, characterised in that the plants have been transformed with and express the coding regions of a polynucleotide comprising a sequence encoding a phytoene desaturase.

19. A method according to claim 18 wherein the polynucleotide is that mentioned in any one of claims 2 to 12.

20. A method according to either of claims 18 or 19, wherein the said herbicide is selected from the group consisting of, isoxaflutole, diketonitriles such as 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-CF_3-phenyl)propane-1,3-dione and 2-cyano-3-cyclopropyl-1-(2-SO_2CH_3-4-2,3Cl_2phenyl)propane-1,3-dione, triketones such as sulcotrione, and mesotrione (BSI-proposed), pyrazolynate and pyrazoxyfen.

21. A method according to any one of claims 18 to 20, wherein the field is treated with a pesticide selected from the group consisting of a fungicide, insecticide and nematicide, either prior to or post application to the field of the herbicide.

FIGURE 1



SEQUENCE LISTING

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ctg ctt gaa caa cgt gat aaa ccc ggc ggt cgg gct tat gtc tac gag   146
Leu Leu Glu Gln Arg Asp Lys Pro Gly Gly Arg Ala Tyr Val Tyr Glu
     30                  35                  40

gat cag ggg ttt acc ttt gat gca ggc ccg acg gtt atc acc gat ccc   194
Asp Gln Gly Phe Thr Phe Asp Ala Gly Pro Thr Val Ile Thr Asp Pro
 45                  50                  55                  60

agt gcc att gaa gaa ctg ttt gca ctg gca gga aaa cag tta aaa gag   242
Ser Ala Ile Glu Glu Leu Phe Ala Leu Ala Gly Lys Gln Leu Lys Glu
                 65                  70                  75

tat gtc gaa ctg ctg ccg gtt acg ccg ttt tac cgc ctg tgt tgg gag   290
Tyr Val Glu Leu Leu Pro Val Thr Pro Phe Tyr Arg Leu Cys Trp Glu
             80                  85                  90

tca ggg aag gtc ttt aat tac gat aac gat caa acc cgg ctc gaa gcg   338
Ser Gly Lys Val Phe Asn Tyr Asp Asn Asp Gln Thr Arg Leu Glu Ala
         95                  100                 105

cag att cag cag ttt aat ccc cgc gat gtc gaa ggt tat cgt cag ttt   386
Gln Ile Gln Gln Phe Asn Pro Arg Asp Val Glu Gly Tyr Arg Gln Phe
    110                 115                 120

ctg gac tat tca cgc gcg gtg ttt aaa gaa ggc tat cta aag ctc ggt   434
Leu Asp Tyr Ser Arg Ala Val Phe Lys Glu Gly Tyr Leu Lys Leu Gly
125                 130                 135                 140
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act gtc cct ttt tta tcg ttc aga gac atg ctt cgc gcc gca cct caa   482
Thr Val Pro Phe Leu Ser Phe Arg Asp Met Leu Arg Ala Ala Pro Gln
                145                 150                 155

ctg gcg aaa ctg cag gca tgg aga agc gtt tac agt aag gtt gcc agt   530
Leu Ala Lys Leu Gln Ala Trp Arg Ser Val Tyr Ser Lys Val Ala Ser
            160                 165                 170

tac atc gaa gat gaa cat ctg cgc cag gcg ttt tct ttc cac tcg ctg   578
Tyr Ile Glu Asp Glu His Leu Arg Gln Ala Phe Ser Phe His Ser Leu
        175                 180                 185

ttg gtg ggc ggc aat ccc ttc gcc acc tca tcc att tat acg ttg ata   626
Leu Val Gly Gly Asn Pro Phe Ala Thr Ser Ser Ile Tyr Thr Leu Ile
    190                 195                 200

cac gcg ctg gag cgt gag tgg ggc gtc tgg ttt ccg cgt ggc ggc acc   674
His Ala Leu Glu Arg Glu Trp Gly Val Trp Phe Pro Arg Gly Gly Thr
205                 210                 215                 220

ggc gca tta gtt cag ggg atg ata aag ctg ttt cag gat ctg ggt ggc   722
Gly Ala Leu Val Gln Gly Met Ile Lys Leu Phe Gln Asp Leu Gly Gly
                225                 230                 235

gaa gtc gtg tta aac gcc aga gtc agc cat atg gaa acg aca gga aac   770
Glu Val Val Leu Asn Ala Arg Val Ser His Met Glu Thr Thr Gly Asn
            240                 245                 250

aag att gaa gcc gtg cat tta gag gac ggt cgc agg ttc ctg acg caa   818
Lys Ile Glu Ala Val His Leu Glu Asp Gly Arg Arg Phe Leu Thr Gln
        255                 260                 265

gcc gtc gcg tca aat gca gat gtg gtt cat acc tat cgc gac ctg tta   866
Ala Val Ala Ser Asn Ala Asp Val Val His Thr Tyr Arg Asp Leu Leu
    270                 275                 280

agc cag cac cct gcc gcg gtt aag cag tcc aac aaa ctg cag act aag   914
Ser Gln His Pro Ala Ala Val Lys Gln Ser Asn Lys Leu Gln Thr Lys
285                 290                 295                 300

cgc atg agt aac tct ctg ttt gtg ctc tat ttt ggt ttg aat cac cat   962
Arg Met Ser Asn Ser Leu Phe Val Leu Tyr Phe Gly Leu Asn His His
                305                 310                 315

cat gat cag ctc gcg cat cac acg gtt tgt ttc ggc ccg cgt tac cgc  1010
His Asp Gln Leu Ala His His Thr Val Cys Phe Gly Pro Arg Tyr Arg
            320                 325                 330

gag ctg att gac gaa att ttt aat cat gat ggc ctc gca gag gac ttc  1058
Glu Leu Ile Asp Glu Ile Phe Asn His Asp Gly Leu Ala Glu Asp Phe
        335                 340                 345

tca ctt tat ctg cac gcg ccc tgt gtc acg gat tcg tca ctg gcg cct  1106
Ser Leu Tyr Leu His Ala Pro Cys Val Thr Asp Ser Ser Leu Ala Pro
    350                 355                 360

gaa ggt tgc ggc agt tac tat gtg ttg gcg ccg gtg ccg cat tta ggc  1154
Glu Gly Cys Gly Ser Tyr Tyr Val Leu Ala Pro Val Pro His Leu Gly
365                 370                 375                 380

acc gcg aac ctc gac tgg acg gtt gag ggg cca aaa cta cgc gac cgt  1202
Thr Ala Asn Leu Asp Trp Thr Val Glu Gly Pro Lys Leu Arg Asp Arg
                385                 390                 395

att ttt gcg tac ctt gag cag cat tac atg cct ggc tta cgg agt cag  1250
Ile Phe Ala Tyr Leu Glu Gln His Tyr Met Pro Gly Leu Arg Ser Gln
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            400                 405                 410

ctg gtc acg cac cgg atg ttt acg ccg ttt gat ttt cgc gac cag ctt   1298
Leu Val Thr His Arg Met Phe Thr Pro Phe Asp Phe Arg Asp Gln Leu
        415                 420                 425

aat gcc tat cat ggc tca gcc ttt tct gtg gag ccc gtt ctt acc cag   1346
Asn Ala Tyr His Gly Ser Ala Phe Ser Val Glu Pro Val Leu Thr Gln
    430                 435                 440

agc gcc tgg ttt cgg ccg cat aac cgc gat aaa acc att act aat ctc   1394
Ser Ala Trp Phe Arg Pro His Asn Arg Asp Lys Thr Ile Thr Asn Leu
445                 450                 455                 460

tac ctg gtc ggc gca ggc acg cat ccc ggc gca ggc att cct ggc gtc   1442
Tyr Leu Val Gly Ala Gly Thr His Pro Gly Ala Gly Ile Pro Gly Val
                465                 470                 475

atc ggc tcg gca aaa gcg aca gca ggt ttg atg ctg gag gat ctg att   1490
Ile Gly Ser Ala Lys Ala Thr Ala Gly Leu Met Leu Glu Asp Leu Ile
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tga                                                               1493
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Arg Asp Lys Pro Gly Gly Arg Ala Tyr Val Tyr Glu Asp Gln Gly Phe
         35                  40                  45

Thr Phe Asp Ala Gly Pro Thr Val Ile Thr Asp Pro Ser Ala Ile Glu
     50                  55                  60

Glu Leu Phe Ala Leu Ala Gly Lys Gln Leu Lys Glu Tyr Val Glu Leu
 65                  70                  75                  80

Leu Pro Val Thr Pro Phe Tyr Arg Leu Cys Trp Glu Ser Gly Lys Val
                 85                  90                  95

Phe Asn Tyr Asp Asn Asp Gln Thr Arg Leu Glu Ala Gln Ile Gln Gln
            100                 105                 110

Phe Asn Pro Arg Asp Val Glu Gly Tyr Arg Gln Phe Leu Asp Tyr Ser
        115                 120                 125

Arg Ala Val Phe Lys Glu Gly Tyr Leu Lys Leu Gly Thr Val Pro Phe
    130                 135                 140

Leu Ser Phe Arg Asp Met Leu Arg Ala Ala Pro Gln Leu Ala Lys Leu
145                 150                 155                 160

Gln Ala Trp Arg Ser Val Tyr Ser Lys Val Ala Ser Tyr Ile Glu Asp
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Glu His Leu Arg Gln Ala Phe Ser Phe His Ser Leu Leu Val Gly Gly
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            180                 185                 190

Asn Pro Phe Ala Thr Ser Ser Ile Tyr Thr Leu Ile His Ala Leu Glu
        195                 200                 205

Arg Glu Trp Gly Val Trp Phe Pro Arg Gly Gly Thr Gly Ala Leu Val
    210                 215                 220

Gln Gly Met Ile Lys Leu Phe Gln Asp Leu Gly Gly Glu Val Val Leu
225                 230                 235                 240

Asn Ala Arg Val Ser His Met Glu Thr Thr Gly Asn Lys Ile Glu Ala
                245                 250                 255

Val His Leu Glu Asp Gly Arg Arg Phe Leu Thr Gln Ala Val Ala Ser
            260                 265                 270

Asn Ala Asp Val Val His Thr Tyr Arg Asp Leu Leu Ser Gln His Pro
        275                 280                 285

Ala Ala Val Lys Gln Ser Asn Lys Leu Gln Thr Lys Arg Met Ser Asn
    290                 295                 300

Ser Leu Phe Val Leu Tyr Phe Gly Leu Asn His His His Asp Gln Leu
305                 310                 315                 320

Ala His His Thr Val Cys Phe Gly Pro Arg Tyr Arg Glu Leu Ile Asp
                325                 330                 335

Glu Ile Phe Asn His Asp Gly Leu Ala Glu Asp Phe Ser Leu Tyr Leu
            340                 345                 350

His Ala Pro Cys Val Thr Asp Ser Ser Leu Ala Pro Glu Gly Cys Gly
        355                 360                 365

Ser Tyr Tyr Val Leu Ala Pro Val Pro His Leu Gly Thr Ala Asn Leu
    370                 375                 380

Asp Trp Thr Val Glu Gly Pro Lys Leu Arg Asp Arg Ile Phe Ala Tyr
385                 390                 395                 400

Leu Glu Gln His Tyr Met Pro Gly Leu Arg Ser Gln Leu Val Thr His
                405                 410                 415

Arg Met Phe Thr Pro Phe Asp Phe Arg Asp Gln Leu Asn Ala Tyr His
            420                 425                 430

Gly Ser Ala Phe Ser Val Glu Pro Val Leu Thr Gln Ser Ala Trp Phe
        435                 440                 445

Arg Pro His Asn Arg Asp Lys Thr Ile Thr Asn Leu Tyr Leu Val Gly
    450                 455                 460

Ala Gly Thr His Pro Gly Ala Gly Ile Pro Gly Val Ile Gly Ser Ala
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tct agg ggg caa tcc gcc gca gtg gct cca ttc ggc ggc ctc aaa tcc   96
Ser Arg Gly Gln Ser Ala Ala Val Ala Pro Phe Gly Gly Leu Lys Ser
             20                  25                  30

atg act gga ttc cca gtg aag aag gtc aac act gac att act tcc att   144
Met Thr Gly Phe Pro Val Lys Lys Val Asn Thr Asp Ile Thr Ser Ile
         35                  40                  45

aca agc aat ggt gga aga gta aag tgc atg aaa cca act acg gta att   192
Thr Ser Asn Gly Gly Arg Val Lys Cys Met Lys Pro Thr Thr Val Ile
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ggt gca ggc ttc                                                  204
Gly Ala Gly Phe
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Ser Arg Gly Gln Ser Ala Ala Val Ala Pro Phe Gly Gly Leu Lys Ser
             20                  25                  30

Met Thr Gly Phe Pro Val Lys Lys Val Asn Thr Asp Ile Thr Ser Ile
         35                  40                  45

Thr Ser Asn Gly Gly Arg Val Lys Cys Met Lys Pro Thr Thr Val Ile
     50                  55                  60

Gly Ala Gly Phe
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INTERNATIONAL SEARCH REPORT

Interi nal Application No
PCT/GB 99/01059

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 A01H5/00 A01H5/10 //C12N15/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MISAWA N ET AL: "EXPRESSION OF AN ERWINA PHYTOENE DESATURASE GENE NOT ONLY CONFERS MULTIPLE RESISTANCE TO HERBICIDES INTERFERING WITH CAROTENOID BIOSYNTHESIS BUT ALSO ALTERS XANTHOPHYLL METABOLISM IN TRANSGENIC PLANTS" PLANT JOURNAL, vol. 6, no. 4, 1 January 1994 (1994-01-01), pages 481-489, XP002017203 ISSN: 0960-7412 the whole document	15,16
A	page 486, column 1, paragraph 2; table 1	

A	WO 97 27285 A (UNIV ARIZONA) 31 July 1997 (1997-07-31) page 6, line 7-24	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 1999	30/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bilang, J

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 99/01059

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BARTA I C ET AL: "BENZOYLCYCLOHEXANEDIONE HERBICIDES ARE STRONG INHIBITORS OF PURIFIED P-HYDROXYPHENYLPYRUVIC ACID DIOXYGENASE OF MAIZE" PESTICIDE SCIENCE, vol. 45, no. 3, 1 November 1995 (1995-11-01), page 286/287 XP000547268 ISSN: 0031-613X the whole document ---	
A	SCHULZ A ET AL: "SC-0051, A 2-BENZOYL-CYCLOHEXANE-1,3-DIONE BLEACHING HERBICIDE, IS APOTENT INHIBITOR OF THE ENZYME P-HYDROXYPHENYLPYRUVATE DIOXYGENASE" FEBS LETTERS, vol. 318, no. 2, 1 March 1993 (1993-03-01), pages 162-166, XP002028049 ISSN: 0014-5793 abstract ---	
A	BABCZINSKI P ET AL: "SUBSTITUTED TETRAHYDROPYRIMIDINONES. A NEW CLASS OF HERBICIDAL COMPOUNDS INDUCING CHLOROSIS BY INHIBITION OF PHYTOENE DESATURATION" PESTICIDE SCIENCE, vol. 30, no. 3, 1 January 1990 (1990-01-01), pages 339-342, XP000202878 ISSN: 0031-613X the whole document ---	
A	MISAWA ET AL.: "E. uredovora carotenoid biosynthesis genes" EMBL DATABASE ACCESSION NUMBER D90087, 6 January 1991 (1991-01-06), XP002111545 the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ıal Application No
PCT/GB 99/01059

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9727285 A	31-07-1997	AU 1845397 A	20-08-1997
		EP 0877793 A	18-11-1998